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Address for correspondence: Jonas Klingström, Center for Infectious Medicine, Department of Medicine Huddinge, Karolinska Institutet, 141 86 Stockholm, Sweden; email: jonas.klingstrom@ki.se

Human Monkeypox without Viral Prodrome or Sexual Exposure, California, USA, 2022

Abraar Karan, Ashley R. Styczynski, ChunHong Huang, Malaya K. Sahoo, Krithika Srinivasan, Benjamin A. Pinsky, Jorge L. Salinas

Author affiliation: Stanford University School of Medicine, Stanford, California, USA

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We report human monkeypox in a man who returned to the United States from the United Kingdom and reported no sexual contact. He had vesicular and pustular skin lesions but no anogenital involvement. The potential modes of transmission may have implications for the risk of spread and for epidemic control.

The 2022 multicountry monkeypox outbreak has been linked primarily to intimate contact among men who have sex with men (*1*,*2*). We describe a case of monkeypox in a traveler who returned from the United Kingdom to the United States who did not report recent sexual contact.

A man in his 20s sought care at an emergency department in Stanford, California, USA, on day 7 of an asynchronous, diffuse vesicular rash following travel to the United Kingdom. The first lesion appeared ≈14 days after he attended a large, crowded outdoor event at which he had close contact with others, including close dancing, for a few hours. He said that many attendees were in sleeveless tops and shorts. He wore pants and a short-sleeved top. He did not notice any skin lesions on anyone present, nor did he notice anyone who seemed sick. He shared an e-cigarette with a woman that he met while there. The event was not a rave and was not attended specifically or mostly by persons identifying as gay or bisexual. He attended other similar outdoor events over 4 days. He reported consuming alcohol but no other drug use at these events. He did not wear a mask at these events. He had contact with domestic dogs that he petted.

He took 2 flights to return to the United States; masks were worn on 1 flight. He identifies as bisexual but reported no recent sexual contacts during his travels or in the preceding 3 months. He reported no close indoor activities, although he traveled on crowded public trains. He reported no close contacts

Table. Monkeypox virus DNA levels in clinical specimens from a man with monkeypox. California, USA, 2022*

Specimen type	Days since symptom onset	Viral copies/mL	log_{10} copies/mL	Ct value
Lesion swab	7	65,647,690	7.82	12.7
Nasopharyngeal swab	7	1,200	3.08	27.6
Saliva	10	3,030	3.48	26.3
Rectal swab	10	Detected, unable to quantitate	NA	30.2
Conjunctival swab	10	Detected, unable to quantitate	NA	32.6
Oropharyngeal swab	10	Not detected	NA	NA
Semen	10	Not detected	NA	NA

*The concentrations of the lesion, and self-collected rectal and conjunctival swab specimens are expressed in copies/mL phosphate-buffered saline (PBS). These samples, as well as the self-collected oropharyngeal swab, were collected dry and rehydrated with 1 mL PBS. The nasopharyngeal swab was healthcare worker–collected in 3 mL viral transport media. Cycle threshold values and estimated concentrations are based on the results from the non-variola orthopoxvirus quantitative PCR. All samples with values in copies/mL or reported as detected, unable to quantitate, were positive by both the non-variola orthopoxvirus qPCR and the clade 2/3 monkeypox qPCR. Ct, cycle threshold; NA, not applicable.

since his return. He lives with 1 roommate who did not manifest any symptoms. He had a history of syphilis treated 3 months earlier and was taking HIV preexposure prophylaxis. He denied preceding fevers, chills, headache, lymph node swelling, cough, fatigue, or anorectal pain.

We noted multiple nondraining skin lesions at different stages of appearance, including a centrally umbilicated vesicle on his left palm, a crusting flat lesion on his lip, and pustules on his right and left knuckles and on his lateral torso and back. He had no penile, testicular, or anal lesions and no cervical, axillary, or inguinal lymphadenopathy (Appendix, https://wwwnc.cdc.gov/EID/article/28/10/22-1191-App1.pdf).

Results of complete blood count and basic metabolic panel results were unremarkable. Results of rapid HIV-1 antibody/antigen test was negative, as was urine testing for *N. gonorrhoeae* and *C. trachomatis*. Rapid plasma reagin test results were positive (titer of 1:1). The palmar vesicle was unroofed; a swab of the expressed clear fluid tested positive for nonvariola orthopoxvirus DNA by quantitative PCR (qPCR) and was confirmed as monkeypox virus DNA by qPCR specific for clade 2/3 (West Africa) monkeypox (Table; Appendix). A nasopharyngeal swab specimen that tested negative for SARS-CoV-2 was positive for monkeypox virus DNA using this 2-step testing algorithm. We did not prescribe specific monkeypox treatment because the patient did not have complications or risk factors for severe disease.

We performed follow-up monkeypox virus testing with patient consent 3 days after initial evaluation (day 10 after symptom onset) to clarify viral shedding. We detected virus DNA in a saliva sample, as well as from patient-collected conjunctival and rectal swabs using both the non-variola orthopoxvirus and clade 2/3 monkeypox virus qPCRs. Lesions resolved by day 26 after symptom onset.

This patient tested positive for monkeypox virus DNA from several nonlesion samples. The nasopharyngeal and saliva findings are noteworthy because the patient did not report respiratory symptoms. In addition, the detectable viral DNA in the rectal swab specimen in the absence of visible anal lesions or pain indicates a potential for sustained sexual transmission, although the viral DNA levels were low; contamination during self-collection cannot be ruled out. We were unable to assess whether internal rectal lesions were present.

This case highlighted the distinctiveness of clinical manifestations as they indicated potential routes of transmission during the 2022 multicountry outbreak of monkeypox. This patient did not report recent sexual contact, did not have evidence of genital lesions or inguinal lymphadenopathy (*3*), and did not report a viral prodrome. His primary risk factor was close, nonsexual contact with numerous unknown persons at a crowded outdoor event. His case highlights the potential for spread at such gatherings, which may have implications for epidemic control. The lack of both sexual exposure and anogenital involvement indicates that mode of transmission may be associated with clinical symptoms; fomites (hotel bedding and sheets, high-touch areas in public settings) may be alternative modes of transmission. Overall, the viral inoculum required for all possible modes of transmission remains an area of active investigation.

This case also demonstrates the importance of local monkeypox virus testing, rather than centralized testing in public health or commercial reference laboratories. Local testing enabled diagnosis in <12 hours and immediate notification to local and state public health authorities for isolation and contact tracing.

About the Author

Dr. Karan is an infectious disease physician and postdoctoral researcher at Stanford University. His research focuses on infectious disease epidemiology and interventions to slow epidemic spread.

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Address for correspondence: Abraar Karan, Stanford University School of Medicine, 300 Pasteur Dr, Lane 134, Stanford, CA 94304, USA; email: abraar@stanford.edu

Introduction and Differential Diagnosis of Monkeypox in Argentina, 2022

Adrian Lewis, Alejandro Josiowicz, Stella Maris Hirmas Riade, Monica Tous, Gustavo Palacios,[1] Daniel M. Cisterna[1]

Author affiliations: Instituto Nacional de Enfermedades Infecciosas, ANLIS Dr. Carlos G. Malbran, Buenos Aires, Argentina (A. Lewis, A. Josiowicz, S.M.H. Riade, M. Tous, D.M. Cisterna); Icahn School of Medicine at Mount Sinai, New York, New York, USA (G.P.)

We report detection of cases of monkeypox virus infection in Argentina in the context of a marked increase in confounding cases of atypical hand-foot-and-mouth syndrome caused by enterovirus coxsackie A6. We recommend performing an accurate differential virological diagnosis for exanthematous disease in suspected monkeypox cases.

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Global surveillance of monkeypox cases has resulted in the detection of an increasing number of suspected cases in countries to which the disease is not endemic (*1*). We report the results of a virological investigation of 9 suspected cases of monkeypox from Argentina (n = 6) and Bolivia (n = 3) detected during May 22–June 8, 2022. The investigation was conducted using World Health Organization case definitions (*2*).

We attempted laboratory diagnosis for all 9 cases by using classical and molecular methods such as electron microscopy (EM) and conventional orthopoxvirus PCR. We analyzed swab samples collected from the skin, genital lesions, or both for monkeypox screening. We performed negative staining electron microscopy using direct absorption for 10 minutes of a 10-μL sample volume on fomvar-coated 400 mesh grids. We performed staining with 1% phosphotungstic acid (*3*) and examined samples using a Zeiss EM-109 transmission electron microscope.

We extracted viral nucleic acid by using the High Pure Viral RNA kit (Roche Molecular Biochemicals, https://www.roche.com) according to the manufacturer's instructions. We performed end-point PCR amplification by using primers EACP1 and EACP2 targeting the complete viral hemagglutinin gene, as done previously (*4*). We sequenced amplicon PCR fragments by using BigDye Terminator version 3.1 reagent in an ABI3500 Genetic Analyzer automatic sequencer (both ThermoFisher Scientific, https://www.thermofisher.com). We performed phylogenetic analysis by using the maximum-likelihood method and Tamuka 3-parameter model according to Modeltest using MEGA software (https://www.megasoftware.net). We produced bootstraps using 500 replicates. For differential diagnosis, we analyzed negative monkeypox virus (MPXV) samples by molecular methods for the detection of herpes simplex virus, varicella zoster virus, and enterovirus. We performed molecular typing of enteroviruses as previously reported (*5*).

The images obtained by EM in cases 1–3, all from Argentina, showed the presence of viral particles compatible with a member of the genus *Orthopoxvirus* (Appendix Figure 1, https://wwwnc.cdc.gov/EID/article/28/10/22-1075-App1.pdf). The phylogenetic analysis of the complete hemagglutinin genes for these viruses confirmed the identification of MPXV (West African clade) (Appendix Figure 2). Enterovirus was identified by PCR in 4 (66.7%) of the remaining 6 cases (2 from Argentina and 2 from Bolivia). Coxsackievirus A6 (CV-A6) was identified in 3 of these 4 cases. CV-A6 is usually associated with atypical hand-foot-mouth syndrome. Finally, the 6 samples analyzed were negative for herpes simplex virus and varicella zoster virus. In summary, of the 9

[1]These authors were co–principal investigators.

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Appendix

Methods

Lesion, rectal, conjunctival, and oropharyngeal swab samples were placed in conical tubes without transport media (dry) and rehydrated in 1mL phosphate buffered saline at the Stanford Clinical Virology Laboratory. The nasopharyngeal swab was collected in 3 mL viral transport media. Saliva and semen were collected in sterile containers. Total nucleic acids were purified from 400 µL of each sample using the EZ1 Virus Mini Kit version 2.0 on the EZ1 Advanced XL instrument (QIAGEN, https://www.qiagen.com) and eluted in 60 uL of buffer AVE.

Testing for monkeypox virus DNA was performed using 2 laboratory-developed quantitative PCR (qPCR) assays modified from the published work of the US Centers for Disease Control and Prevention (*1*,*2*). The first screening qPCR targets viral DNA polymerase sequences conserved throughout the non-variola orthopoxviruses, including monkeypox. The second confirmatory qPCR targets viral TNF receptor sequences specific for clade 2/3 (West Africa) monkeypox viruses. All samples reported in this manuscript as positive for monkeypox virus DNA were detected by both qPCRs.

These assays were performed using the Luna Universal Probe qPCR Master Mix (New England Biolabs, https://www.neb.com) on the Rotor-Gene Q instrument (QIAGEN). Each reaction was performed using 10 µL of eluate, with a final reaction volume of 25 µL. Primers and FAM-labeled probes were obtained from Integrated DNA Technologies and were added at final concentrations of 300 nM and 100 nM, respectively. As internal control (IC), primers and HEX-labeled probes targeting the human β-globin gene were added in multiplex at final concentrations of 100 nM and 50 nM, respectively (*3*). Cycling conditions were as follows: hold at 94°C for 2 minutes, followed by 45 cycles of 94°C for 15 seconds, 60°C for 30 seconds, and

72°C for 10 seconds. Detection was performed in the green (non-variola orthopoxvirus or clade 2/3 monkeypox) and yellow (IC:β-globin) channels; the threshold was set at 0.05 for both channels. To report a sample as negative for virus DNA, the IC must be amplified at a cycle threshold (Ct) <35 cycles. This ensures adequate specimen collection and nucleic acid extraction, as well as the absence of PCR inhibitors.

The non-variola orthopoxvirus qPCR was calibrated using quantitated double stranded DNA containing the assay target sequence (gBlock; Integrated DNA Technologies, https://www.idtdna.com). The 95% lower limit of detection (LLOD) was determined using serial dilutions of purified nucleic acids from a quantitated high-positive sample and calculated using probit regression. The non-variola orthopoxvirus qPCR has a 95% LLOD of 81 copies/mL (95% CI 59–332 copies/mL). Based on further precision experiments, the lower limit of quantitation (LLOQ) was determined to be 500 copies/mL.

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